REMARKS/ARGUMENTS

The Pending Claims

Claims 1, 7-14, and 17-26 are pending. Pursuant to a restriction requirement, claims 7-14 and claims 21-26 have been withdrawn. Therefore, claims 1 and 17-20 are currently subject to examination.

Amendments to the Claims

Claim 1 has been amended to recite that the control sample is of the central nervous system of another mammal of the same species. This amendment finds support in the specification as filed at, e.g., paragraphs [0065], [0067], and [0075].

No new matter has been added by way of these amendments to the claims.

Discussion of the Enablement Rejection

Claims 1 and 17-20 remain rejected under Section 112, first paragraph, as allegedly lacking enablement. The Examiner has alleged several reasons for the rejection. Each of these reasons is traversed as explained below and as referenced in the 132 Declaration of Nancy E. J. Berman, Ph.D. ("decl.") filed concurrently with this response.

The USPTO makes several allegations regarding the macaque monkey model system used in the present application. The model system study of the Examples shows upregulation of Cripto-1 is indicative of the presence of NeuroAIDS. However, the USPTO alleges that (i) the study only used a single control and, essentially, the study cannot be used to determine indication of a disease due to a small control sample size, (ii) post-exsanguination collection of tissue cannot be used to reliably determine gene expression, (iii) the model system detected the macaque homologue of the human sequence (SEQ ID NO: 1) of Cripto-1 and would not detect human Cripto-1, (iv) there were no studies performed in humans, (v) the macaque monkey is not a reasonable model for humans and SHIV infection is not a reasonable model for NeuroAIDS, (vi) there is no nexus for the role of increased SEQ ID NO: 1 and NeuroAIDS, (vii) Raghaven et al., Brain Pathol., 7:851-861 (1997) ("Raghaven") teaches SHIV has different affects on two members of the macaque family and that SIV- and SHIV-induced NeuroAIDS have different pathologies, and (viii) Enard et al., Science,

296:340-343 (2002) ("Enard") teaches intra- and interspecies variation in gene expression in brain tissue is substantial and Cheung et al., Nat. Genet., 33:422-425 (2003) ("Cheung") teaches there is natural variation in gene expression among different individuals and renders unpredictable any correlation between altered gene expression and phenotype.

The USPTO also makes several allegations regarding the methods of detection. The Examples show that upregulation of Cripto-1 in the central nervous system is indicative of the presence of NeuroAIDS. However, the USPTO alleges that (i) knowledge of the particular housekeeping genes used in a gene expression study is required, (ii) Vandesompele et al., Genome Biol., 3:1-11 (2002) ("Vandesompele") teaches at least three housekeeping genes are required for accurate normalization of gene expression data and that using one gene for normalization is not reliable due to large variation in housekeeping gene expression, (iii) Wu, J. Pathol., 195: 53-65 (2001) ("Wu") teaches that conclusions drawn from gene expression data depend heavily on the particular choice of data analysis, and (iv) Newton et al., J. Comput. Biol., 8:37-52 (2001) ("Newton") teaches that a basic statistical problem is determining when the measured differential expression is likely to reflect a real biological shift in gene expression, and replication of data is critical to validation.

As Dr. Berman explains (decl. at ¶ 6), macaque monkey studies are necessarily constrained by expense and housing considerations. Individual macaque monkeys are much more expensive than individual mice, rats, etc. used for animal model studies. Also, macaque monkeys require more housing space than, e.g., mice or rats, and require more specialized facilities than those necessary for these mammals. The specialized facilities, then, also further increase the cost of performing macaque studies compared to performing studies on other mammals commonly used for animal model studies. Due to the expense and specialized facilities required, it was accepted in the art at the time of filing the present application that macaque monkey studies were necessarily on a smaller scale than studies with, e.g., mice or rats. Although other macaque studies have used higher numbers of controls, such studies were still routinely performed on a small number of test macaque monkeys, including using one macaque monkey as a control.

According to Dr. Berman (decl. at ¶ 7), post-exanguination collection of tissue is a reliable method for collecting tissue samples for gene expression analysis. Although post-

exanguination may produce unreliable gene expression data if it is performed incorrectly, the method was an accepted practice in animal model studies at the time of filing the present application and provides reliable results when performed carefully. For the present application, the post-exanguination collection of tissue was performed carefully to ensure reliable results.

As Dr. Berman additionally explains (decl. at ¶ 8), one of ordinary skill in the art would have recognized at the time of filing the present application that primers derived from the human sequence of Cripto-1 (SEQ ID NO: 1) would identify gene expression of macaque Cripto-1 in a macaque animal model. When the macaque studies described in the present application were performed, the expressed gene, identified by human-derived primers, was cloned and identified as macaque Cripto-1. Although macaque and human Cripto-1 are not identical, for the reasons set forth below, one of ordinary skill in the art would have recognized at the time of filing the present application that identification of macaque Cripto-1 in a macaque NeuroAIDS animal model would have been indicative of identification of human Cripto-1 (SEQ ID NO: 1) in a human patient suffering from NeuroAIDS since the macaque monkey animal model was an acceptable animal model of human NeuroAIDS.

Furthermore, according to Dr. Berman (decl. at ¶ 9), studies of altered gene expression in humans presents many obstacles that are overcome when using animal models. For example, NeuroAIDS may be induced in animals to provide specific research conditions; and should more studies be needed, more animals may be induced to develop NeuroAIDS. Also, the extraction of central nervous tissue from humans should not be performed unless absolutely necessary, e.g., after the development of a reliable assay in an animal model, such as that described in the present application. Prior to the development of such an assay, extraction of central nervous tissue would be overly invasive and would not likely be justified.

Moreover, according to Dr. Berman (decl. at ¶ 10), at the time of filing the present application, the macaque monkey animal model was an acceptable animal model for studying NeuroAIDS in humans. The results described in the Examples of the present application were published, after filing the application, as Stephens et al., Neurosci. Lett., 410:94-99 (2006) ("Stephens"). The methods used in the present application and in Stephens also were

used in Buch et al., J. Neuroimmunol., 157:71-80 (2004) ("Buch") and Sui et al., J. Med. Primatol., 32:229-239 (2003) ("Sui"), which were published at around the same time as the filing of the present application. These studies used macaque monkeys to investigate gene expression in brain tissue after induction of HIV-related encephalitis.

Further support is found in the literature that one of ordinary skill in the art would have recognized that, at the time of filing the present application, the SHIV macaque animal model was an acceptable animal model for studying human NeuroAIDS. For example, Buch states "Macaques infected with SIV and SHIV viruses have provided excellent working models for studying mechanisms of the human disease" (abstract). Also, Williams et al., J. Neurovirol., 14:292-300 (2008) ("Williams") is a review article describing SIV and SHIV macaque model systems. Williams states,

> Although SIV-infected macaques have been critical in our understanding of lentiviral neuropathogenesis, the genetic relatedness of these viruses in more to HIV-2 than HIV-1, especially in the *env* gene, which raises the question of whether these models accurately mimic the neuropathogenesis of HIV-1 infection. The construction of the chimeric of SHIV has led to significant advances in the field of AIDS, particularly in the study of immune response and vaccine development.

(page 295, first and second columns, bridging paragraph).

Williams also states, "Such innovation [development of pathogenic SHIV by passaging] has led to the development of several strains and variations of SHIV, each one with specific characteristics in regard to pathogenic phenotype that make it convenient for studying certain aspects of AIDS" (*Id.*), "SHIV infection in macaques creates a model system closely resembling the disease course of human AIDS" (page 295, second column, first full paragraph), and

> Another key feature of the SHIV/macaque model is that the pathology of certain tissues and organ systems, *such as the CNS*, are compatible with the corresponding pathological changes seen in human AIDS. This underscores the utility of SHIV in examining specific HIV-1-induced disease pathology, such as encephalitis, making SHIV an essential model for the study of neuro AIDS [citing Joag, Microbes. Infect., 2:223-229 (2000)]

(page 295, first and second columns, bridging paragraph, emphasis added).

Therefore, Williams describes the SHIV macaque model as an essential model to study human NeuroAIDS. Also, it is noted that Williams here cites as support a paper

published in 2000. Indeed, the section of "SHIV infection in macaques," starting on page 295, cites a majority of studies performed before or around the time of the filing date of the present application. One of the articles cited in William that post-dates the filing of the present application is Stephens, which describes the studies performed for the present application. Therefore, one of ordinary skill in the art at the time the present application was filed would have recognized that the SHIV macaque model for human NeuroAIDS was an acceptable model for studying human pathology associated with NeuroAIDS.

As Dr. Berman explains (decl. at ¶ 11), the function of a gene or its role in a disease-state need not be known for alteration of its expression to indicate the existence of that disease-state. Therefore, even though no nexus is provided between a particular gene and a disease-state, the altered expression of the gene may be indicative of the presence of the disease-state. As was shown in the present application, upregulation of Cripto-1 in nervous tissue is indicative of NeuroAIDS.

According to Dr. Berman (decl. at ¶ 10, and above), the SHIV macaque model is an acceptable model for human NeuroAIDS and a better model than SIV infected macaques. Therefore, that SIV and SHIV infection of macaques manifest in different pathologies is of no consequence since the studies of the present application used an acceptable model, the SHIV macaque model. Furthermore, as Dr. Berman describes (decl. at ¶ 12), although the neuropathogenesis of SHIV in pig-tailed and rhesus macaques may be different, this difference, as described in Raghaven, is due to activation of the virus in rhesus macaque versus pig-tailed macaque. Raghaven states, "SHIV was neuroinvasive in both species and the viral genome was found in several regions of the brains of both pig-tailed and rhesus macaques" (page 858, second column, first full paragraph). Raghaven is silent as to whether the activation or non-activation of SHIV alters Cripto-1 expression. Therefore, non-activation of SHIV in pig-tailed macaque as described in Raghaven does not in itself foreclose the possibility of upregulation of Cripto-1. Also, the SHIV used in the present application is SHIV 50OLNV, a SHIV strain that, as shown in the present application, is activated in pig-tailed macaques (see Example 1 of the present application).

As stated by Dr. Berman (decl. at ¶ 13), intra- and interspecies variations, as described in Enard and Cheung, certainly exist. However, one of ordinary skill in the art would have

recognized at the time of filing the present application that the SHIV macaque model was an acceptable model to model the effects of NeuroAIDS in humans. Therefore, one of ordinary skill in the art would have recognized that the altered gene expression observed in the SHIV macaque models used in the present application is indicative of altered gene expression due to NeuroAIDS in humans.

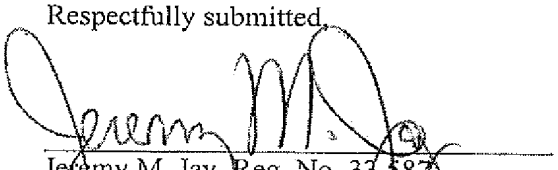
As Dr. Berman explains (decl. at ¶ 14), the methods of detection used in the present application were the accepted methods of ordinary artisans at the time of filing the present application. First, one of ordinary skill in the art would have easily identified housekeeping genes for use in gene expression studies such as the ones described in the present application. Housekeeping genes are defined as those that do not have altered expression under the experimental conditions tested. Therefore, identification of which particular housekeeping genes were used in any gene expression study is not necessary. Furthermore, other papers published around the time of the filing of the present application (Buch and Sui) also used methods similar to those of the present application to determine altered gene expression in macaque model systems. Additionally, the methods of Wu and Newton describe microarray experiments, particularly Affymetrix microarrays. Further, Dr. Berman was not aware of any macaque Affymetrix microarray system that was available when the studies of the present application were performed since the macaque genome had not been sequenced. Therefore, the methods described in these articles did not represent the commonly used practice in the art (for macaque model systems) at the time of filing the present application and thus would have been inappropriate to analyze the data of the present application. Additionally, as Dr. Berman explains, the CLOTECH software used in Newton normalizes the microarray to a set of nine housekeeping genes. However, using a single housekeeping gene for RT-PCR replication of microarray data is standard practice. Finally, Dr. Berman states that regarding Vandesompele, the procedure recommended in this paper has not become standard practice. Therefore, although Vandesompele, Wu, and Newton caution against particular methods of data analysis, one of ordinary skill in the art at the time the present application was filed would have recognized that the methods used in the present application were those accepted in the art.

For the foregoing reasons, the present application enables methods of detecting NeuroAIDS in a mammal by assaying the expression level of Cripto-1. Withdrawal of this rejection is respectfully requested.

Conclusion

Applicants respectfully submit that the patent application is in condition for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,



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